

## Genetic Differentiation Between Subspecies of *Euphydryas phaeton* (Nymphalidae: Nymphalinae)

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### Introduction

The checkerspot butterfly *Euphydryas phaeton* inhabits eastern North America from the maritime provinces of Canada south to Georgia and west to Missouri (Masters, 1968; Bauer, 1975). It is the only species of the genus that occurs in this region, and thus, represents a biogeographic pattern different from that of its congeners in the west, which have ranges that are generally overlapping and in some cases of limited extent. Although *E. phaeton* is clearly distinct from the western species and does not show the extreme phenotypic variation that some of them do, two subspecies have been described. *Euphydryas phaeton phaeton* (Drury) occurs in the northern portion of the species' range where it typically inhabits marshy meadows and similar moist habitats favored by its larval foodplant *Chelone glabra* (Scrophulariaceae); *E. p. ozarkae* (Masters) occurs to the south and southwest and favors drier upland forested habitats where it reportedly feeds on *Gerardia* (= *Aureolaria*: Scrophulariaceae) (Masters, 1968). Bauer (1975) reports that *E. p. ozarkae* feeds on *Lonicera* and that larvae from eggs deposited on *Lonicera* die when transferred to *Chelone*, and those from *Chelone* die when placed on *Lonicera*. He suggests that this larval foodplant intolerance be used as a basis for dividing the taxa. D. Bowers (personal communication) feels Bauer (1975) is in error; she reports that *E. p. ozarkae* feeds naturally on *Gerardia* spp., although both it and *E. p. phaeton* will accept *Lonicera* and survive on it. Furthermore, *E. p. phaeton* can be reared equally well on *Gerardia* or *Chelone*, but *E. p. ozarkae* does significantly better on *Gerardia*. *Gerardia*-feeding populations apparently also occur in upland habitats in some areas of New York state (Shapiro, 1975).

Although these two recognizable groups of populations are most often treated as subspecies, the marked ecological differences between them and the apparent overlap in their geographic ranges suggests the possibility that they may be sibling species.

Here we report the results of our study of genetic differentiation between

*E. p. ozarkae* from Missouri and *E. p. phaeton* from central New York.

### Materials and Methods

Samples of *Euphydryas phaeton* were collected in the summer of 1982 from three areas in central New York and a single area in eastern Missouri. The New York collections were made near Slaterville Springs, Tompkins Co. (N=33); at the Oneonta Airport, Otsego Co. (N=30); and near Milford, Otsego Co. (N=26). The Missouri collection (N=28) was made at Merramec State Park, Franklin Co. The New York populations inhabited wet meadows; the Missouri population inhabited mesic woodland. All butterflies collected were stored in liquid nitrogen prior to electrophoretic analysis.

Allozyme variation was assayed at 25 presumptive gene loci, following the methods of May et. al. (1979). Details of electrophoretic methods and a table of electromorph frequencies are available from ATV on request. Electromorphic frequencies were calculated from direct counts of the electrophoretic phenotypes. Nei's (1972) measure of genetic similarity was used to quantify genetic differentiation between populations.

### Results

There are very few differences in electromorph frequencies among the 3 New York and 1 Missouri populations of *E. phaeton* we examined. The average heterozygosity per locus is  $0.116 \pm 0.019$  (mean  $\pm$  S.E.) and the proportion of polymorphic loci is 0.80. Log-likelihood tests for heterogeneity in electromorph frequencies at each of the 25 loci (Sokal and Rohlf, 1981) illustrate the fundamental genetic similarity among the four populations. At only one locus (MPI) is there a heterogeneity significant at the  $p=0.05$  level, and one expects to find such heterogeneity at the 0.05 level incorrectly in one in 20 such tests.

The genetic identities (Nei, 1972) further illustrate the similarities among the populations (Table 1). The three New York populations attributed to *E. p. phaeton* are somewhat more similar to each other (ave.  $I=0.989$ ) than any of them is to the Missouri population attributed to *E. p. ozarkae* (ave.  $I=0.967$ ), although all four populations are quite similar. The average genetic identity between *E. p. phaeton* and *E. p. ozarkae* that we report here is slightly less than that reported by Brussard et al. (1985), although their value (ave.  $I=0.991$ ) was determined by electrophoresis of some of the same specimens. The discrepancy is due to a number of factors. We examined more specimens, especially of *E. p. phaeton*, but we used only 25 loci rather than the 28 they used. We felt on our further analysis that we could not score all loci with confidence. We also made some minor changes in scoring some of the loci we retained. All of these changes are minor, and none alters the conclusions made in the earlier work.

Table 1. Nei (1972) genetic identities and their standard errors (in parentheses) between three populations of *E. p. phaeton* from New York and one population of *E. p. ozarkae* from Missouri. Nei's index has a value of 1.0 for two populations that share all alleles at the same frequency, and a value of 0.0 for two populations that have no alleles in common. Abbreviations for the localities are as follows: MO = Merramec State Park, MO; NY1 = Slaterville Springs, NY; NY2 = Oneonta, NY; NY3 = Milford, NY.

	NY1	NY2	NY3
MO	0.977(0.018)	0.968(0.024)	0.956(0.033)
NY1	—	0.990(0.004)	0.989(0.006)
NY2	—	—	0.988(0.006)

## Discussion

Lack of differentiation at allozyme loci does not preclude the possibility that the populations in question are reproductively isolated and therefore "good" species; in the absence of other evidence that isolation exists, however, it seems very unlikely that populations that are genetically so similar represent separate species. Sibling species in Lepidoptera for which data are available are clearly more different than these populations of *E. phaeton*. Angevine and Brussard (1979) analyzed differentiation at allozyme loci in populations of the satyrine butterflies *Lethe eurydice* and *L. appalachia* that fly in dissimilar but adjacent habitats within a few meters of each other. Although these *Lethe* species are morphologically nearly indistinguishable, the genetic similarity between them was  $I=0.865$ . Furthermore, although there were no diagnostic loci (i.e. one population fixed for an electromorph that does not occur in the other population), there were significant differences in electromorph frequencies at 5 of the 8 loci examined, and 4 of these were highly significant. Within the genus *Euphydryas*, sibling species are also genetically more distant from each other than are *E. p. phaeton* and *E. p. ozarkae*. The average genetic identity between *E. editha* and its two sibling species *E. chalcedona* and *E. anicia* is reported by Brussard et. al. (1985) to be  $I=0.837$ , and *Euphydryas chalcedona* and *E. anicia*, considered by those authors to be semi-species, have a genetic identity of  $I=0.858$ . (Here we are following the conservative nomenclature of Bauer (1975) rather than that of Miller and Brown (1981), since there are no justifiable reasons to separate North American *Euphydryas* into three separate genera (see Brussard et. al., 1985)). Non-sibling species of butterflies are even more distinct: within the genus *Euphydryas* average between-species identity is only  $I=0.674$  (Brussard et. al., 1985); and among European pierids it is  $I=0.728$  (Geiger, 1980).

Butterfly subspecies are on the average much more similar to each other than are sibling species. Table 2 shows genetic identities between subspecies in 3 genera of butterflies. All are high, most above  $I=0.950$ ; and some (e.g., *napi-bryoniae* complex in *Pieris*) are probably not meaningfully different from unity. These subspecies, therefore, though recognizable on morphological or ecological grounds, and perhaps geographically distant from conspecific populations, are often genetically as similar as local populations. Brittnacher et. al. (1978) suggested that the availability of many visually discernible characters in Lepidoptera makes it easy to find morphological differences among local populations and to elevate some of these to races or subspecies. This may account for the low level of genetic differentiation detected among butterfly subspecies compared to that detected in *Drosophila*.

There are a number of visible phenetic or morphological differences between *E. p. phaeton* and *E. p. ozarkae*. The latter is larger and has reduced orange marginal markings on the ventral side of the wings. There are also the pronounced ecological differences in habitat and foodplant choice. Nonetheless, our analysis of allozymes reveals very little genetic difference among the populations we have examined, even though they are more than 1000 km apart. The lack of concordance between the ecological and morphological traits on the one hand and the electrophoretic traits on the other is not surprising. Singer (1982, 1983) has described variation in host plant preference among and within populations of *E. editha*, and has suggested how shifts in host plant use may evolve. Under strong selection, this evolution may occur relatively quickly. The comparatively slight allozyme differences, however, may have resulted from much weaker selection or none at all, and may indicate that the two lineages have been separate for only a short time. Such would be the case if, as a growing body of evidence now suggests (Wilson et. al., 1977; Thorpe, 1982), allozyme differences accumulate at a stochastically constant rate and thus may serve as a molecular evolutionary clock.

In summary, our results do not provide a definitive answer to the ques-

Table 2. Average genetic identities (Nei, 1972) between subspecies of butterflies.

Species	I	Reference
<i>Pieris napi-bryoniae</i>	0.992	Geiger, 1980
<i>Speyeria callipe</i>	0.983	Brittnacher et. al., 1978
<i>S. coronis</i>	0.929	Brittnacher et. al., 1978
<i>S. zerene</i>	0.982	Brittnacher et. al., 1978
<i>Euphydryas editha</i>	0.970	Brussard et. al., 1985
<i>E. anicia</i>	0.964	Brussard et. al., 1985
<i>F. chalcedona</i>	0.922	Brussard et. al., 1985
<i>E. phaeton</i>	0.967	This study

tion of the appropriate status of *E. p. phaeton* and *E. p. ozarkae*. Overall, there appears to have been little genetic differentiation between the two; however, the striking behavioral and ecological differences remain. Additional evidence from the field on the geographic distribution of the two types of populations and laboratory studies of degrees of interfertility would help to resolve this question.

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